

Creating a Pathway for the Biosynthesis of 1,2,4-Butanetriol

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Collaborators:

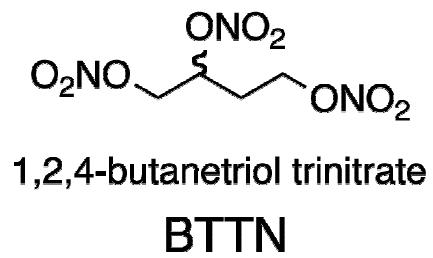
Wei Niu
Man Kit Lau
Mapitso Molefe



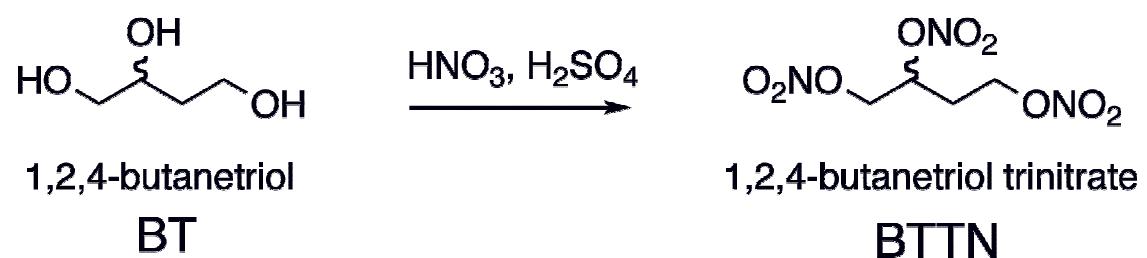
Okemos, Michigan
Minneapolis, Minnesota

Research Areas:

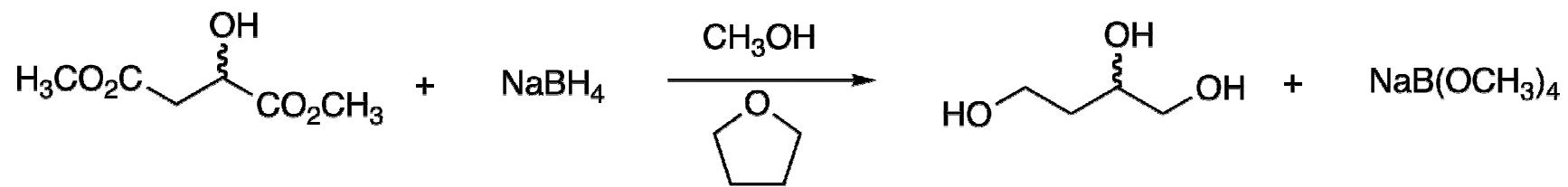
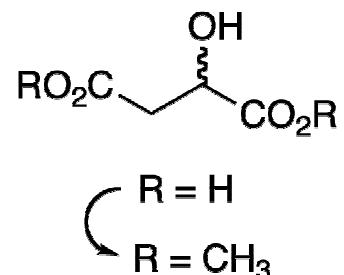
- synthetic chemistry
- catalysis
- materials science
- biocatalysis
- metabolic engineering



- less volatile than nitroglycerine (NG)
- more thermally stable than NG
- lower shock sensitivity relative to NG

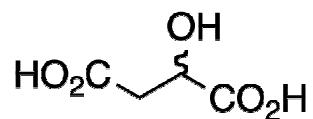


BT Manufacture: Stoichiometric Reduction



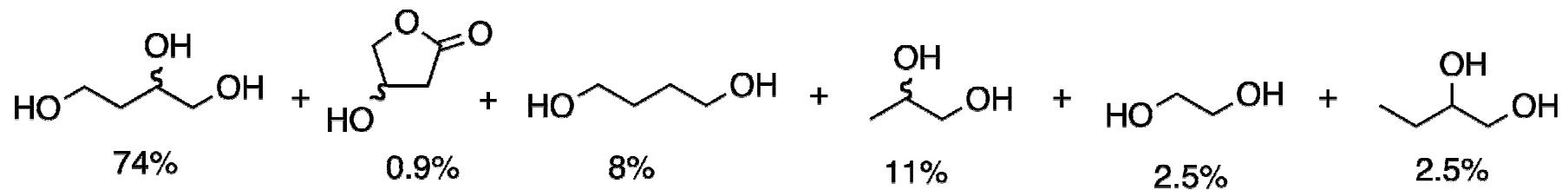
For every ton of BT produced, 2-6 tons of byproduct borate salts are generated.
(WO 98/08793).

BT Synthesis: Catalytic Reduction

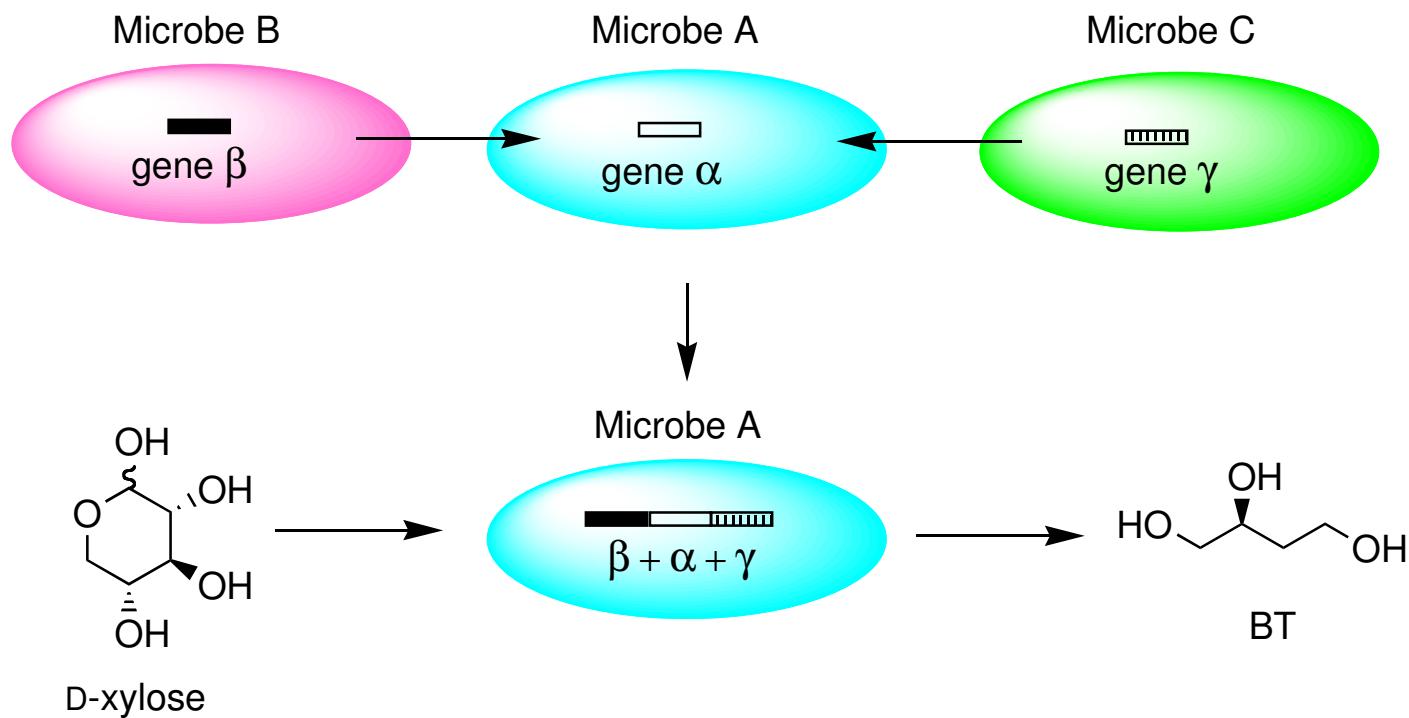


malic acid (1 M)

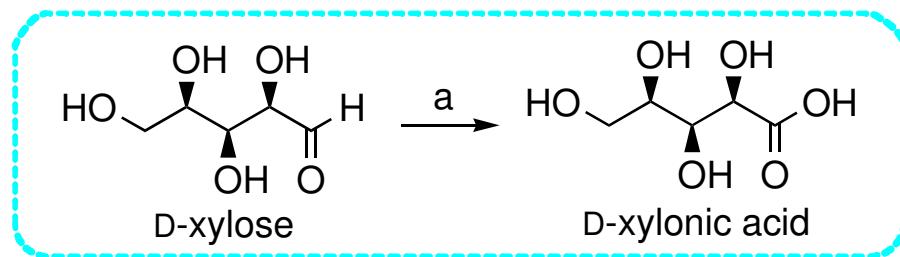
Ru on C
H₂O ↓
5000 psi H₂
135 °C



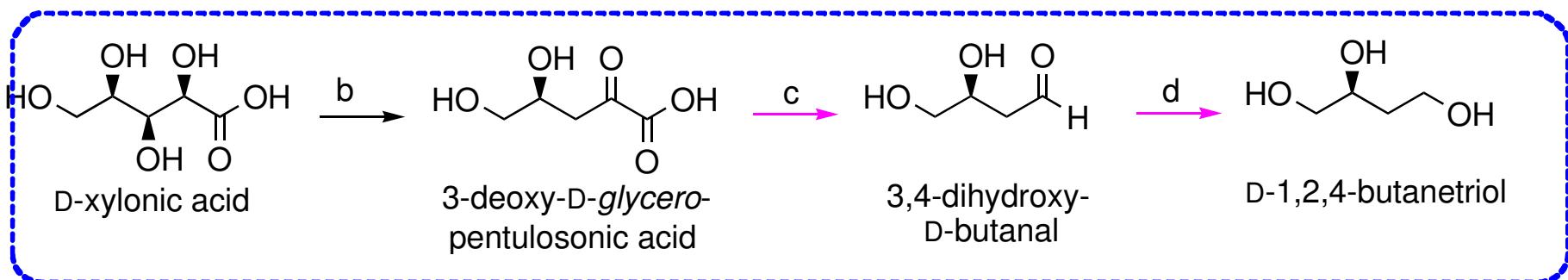
Creation of a Microbial Catalyst



Microbial Synthesis of D-BT



Pseudomonas fragi ATCC 4973 70% Yield



E. coli DH5 α /pWN6.186A 25% Yield

- a. D-xylonate dehydrogenase (*P. fragi*); b. D-xylonate dehydratase (*E. coli*);
- c. benzoylformate decarboxylase (*P. putida*); d. alcohol dehydrogenase (*E. coli*)

→ enzymes catalyzing reactions on native substrates

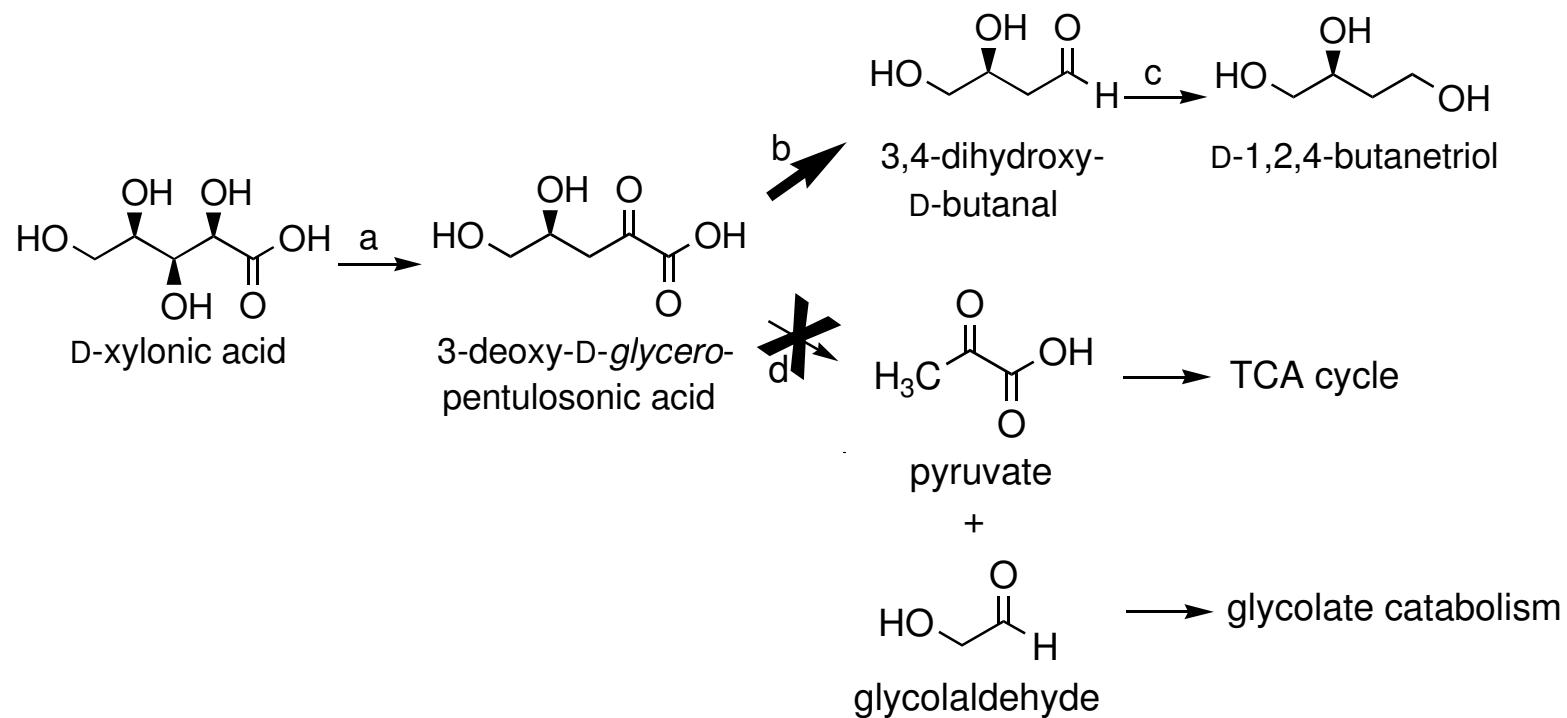
→ enzymes catalyzing reactions on non-native substrates

Escherichia coli Synthesis of D-1,2,4-Butanetriol from D-Xyloonic Acid in Rich Medium and Minimal Salts Medium

host strain	plasmid and encoding genes	1,2,4-BT (g/L)	yield (mol%)	culture medium and carbon source
DH5 α	pWN6.186A P_{tac} - <i>mdlC</i> , Kan ^R , <i>lacI</i> ^Q	1.6	25	rich medium ^a and glucose ^c
W3110 serA	pWN7.126B P_{tac} - <i>mdlC</i> , <i>serA</i> , <i>lacI</i> ^Q	0.020	0.3	minimal medium ^b and glucose ^c

^a tryptone (20 g/L), yeast extract (10 g/L), NaCl (5 g/L), K₂HPO₄ (3.75 g/L); ^b K₂HPO₄ (7.5 g/L), ammonium iron citrate (III) (0.3 g/L), citric acid monohydrate (2.1 g/L); ^c when cell growth entered mid-log phase, IPTG and D-xyloonic acid were added into the culture medium.

Escherichia coli Biosynthesis of D-1,2,4-Butanetriol from D-Xyloonic Acid



- a. D-xylonate dehydratase (*E. coli*); b. benzoylformate decarboxylase (*P. putida*);
 c. alcohol dehydrogenase (*E. coli*); d. aldolase (*E. coli*).

Purification of *Pseudomonas fragi* D-Xylose Dehydratase

205

116

97

66

← ~ 60 kDa

45

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

29

	total protein (mg)	total activity (units)	specific activity (unit/mg)	yield (%)	purification fold
cell lysate	7274	215	0.03	100	1.0
DE-52	527	76	0.14	35	4.8
hydroxyapatite	219	66	0.30	31	10
phenylsepharose	24	50	2.10	23	70
resource Q	11	30	2.91	14	97

N-Terminal Sequences of Trypsin-Digested *Pseudomonas fragi* D-Xylose Dehydratase

estimated position ^a	amino acid sequence	degenerate primers
1	TDST <u>TPKRGRAQL</u> ^b	JWF1006-JWF1009
70	DAGGIPMEFPVHPIAEQSR	N/A
150	IGSGTVLWHAR	N/A
300	HSGVELS <u>LEDWQRVG</u> ^b	JWF1057-JWF1059
400	MSVVGEAFR	N/A
430	AXV <u>FEGPEDYTAR</u> ^b	JWF1004-JWF1005

^a. Positions of peptide fragments were assigned using the positions of the highest scored homologs derived from blasting the NCBI database;

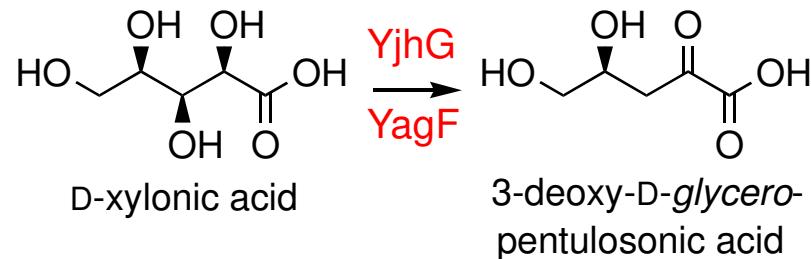
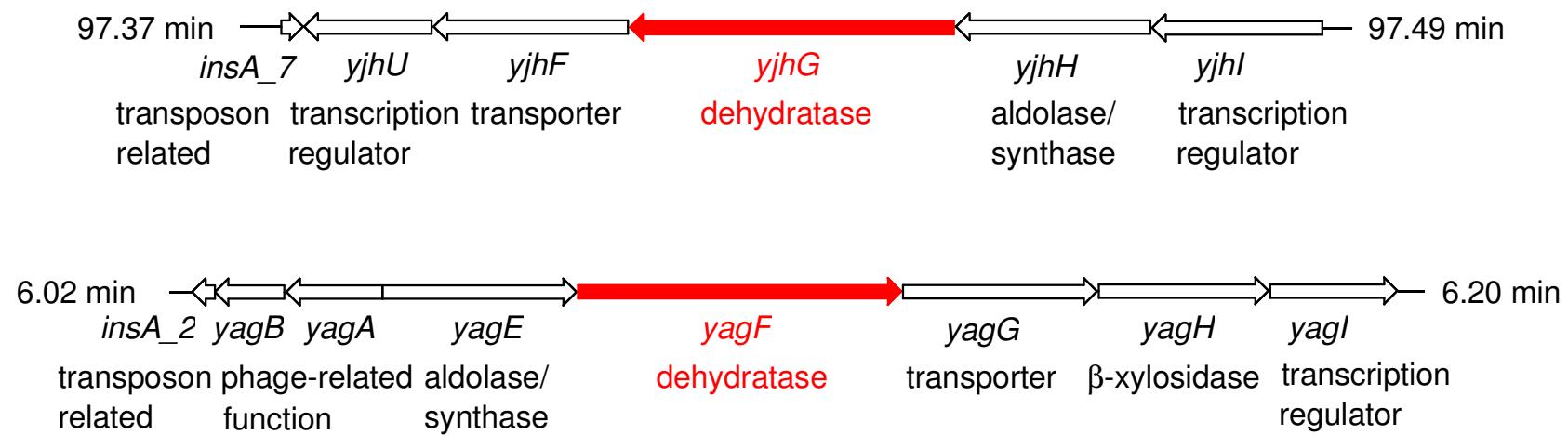
^b. Degenerate primers were designed based on the underlined sequences.

Partial DNA Sequence of *P. fragi* (ATCC 4973) D-Xylose Dehydratase

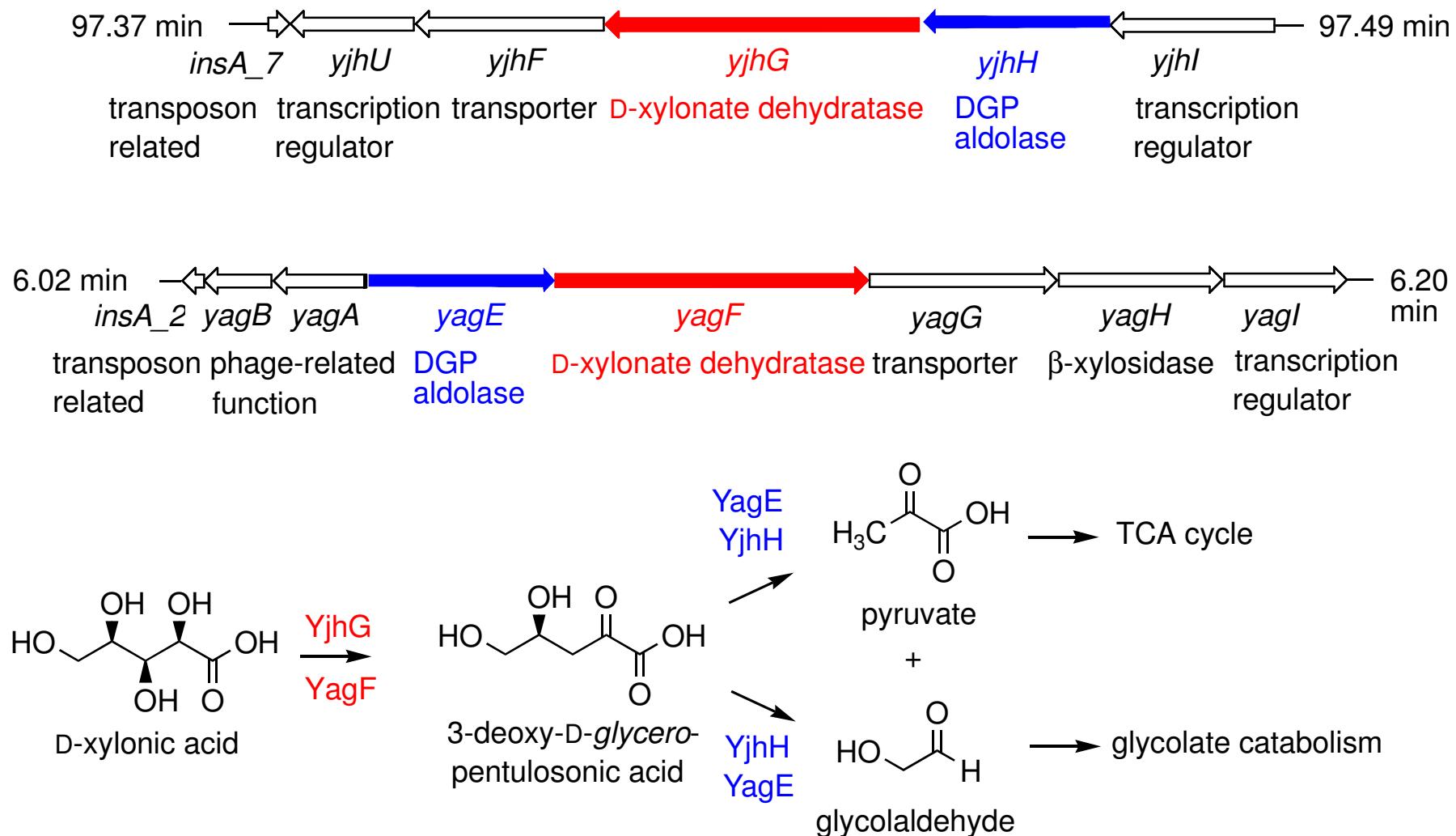
1	CTC	GAG	GAT	TGG	CAG	CGC	GTG	GGT	GAA	GAC	GTG	CCC	TTG	CTG	GTC	AAC	TGC	ATG	CCT	GCC
1	L	E	D	W	Q	R	V	G	E	D	V	P	L	L	V	N	C	M	P	A
61	GGC	GAG	TAC	CTG	GGC	GAA	AGC	TTC	CAC	CGC	GCC	GGT	GGC	GTA	CCG	GCG	GTG	ATG	CAT	GAG
21	G	E	Y	L	G	E	S	F	H	R	A	G	G	V	P	A	V	M	H	E
121	CTG	GAC	AAA	GTG	GGC	CGC	CTG	CAC	CGC	GAT	TGC	CTC	ACG	GTC	AGT	GGC	CGC	AAC	ATG	GGT
41	L	D	K	V	G	R	L	H	R	D	C	L	T	V	S	G	R	N	M	G
181	GAA	GTG	GTC	GCC	GAC	TGC	GTC	ACC	GGC	GAC	CGC	GAC	GTG	ATC	CGC	TCC	TAC	GAA	GAC	CCG
61	E	V	V	A	D	C	V	T	G	D	R	D	V	I	R	S	Y	E	D	P
241	CTG	ATG	CAC	CGC	GCT	GGT	TTT	ATT	GTG	CTC	AGC	GGC	AAC	TTC	TTC	GAC	AGC	GCG	ATC	ATG
81	L	M	H	R	A	G	F	I	V	L	S	G	N	F	F	D	S	A	I	M
301	AAA	ATG	TCG	GTG	GTG	GGC	GAA	GCC	TTC	CGC	AAG	ACC	TAC	CTC	AGC	GAC	CCG	CTG	CAA	CCC
101	K	M	S	V	V	G	E	A	F	R	K	T	Y	L	S	D	P	L	Q	P
361	AAC	AGC	TTC	GAG	GCG	CGG	GCC	ATT	GTG	TTC	GAA	GGC	CCC	GAA	GAC	TAC	AC			
121	N	S	F	E	A	R	A	I	V	F	E	G	P	E	D	Y				

- The DNA sequence was isolated using PCR primer JWF1058 and JWF1004.
- DNA encoding the internal N-terminal sequence at position 400 is colored in red.
- The peptide has homology to hypothetical proteins from *Bradyrhizobium japonicum* USDA 110 and *Burkholderia fungorum* LB400.

Elucidation of the *Escherichia coli* D-Xylynic Acid Catabolic Pathway



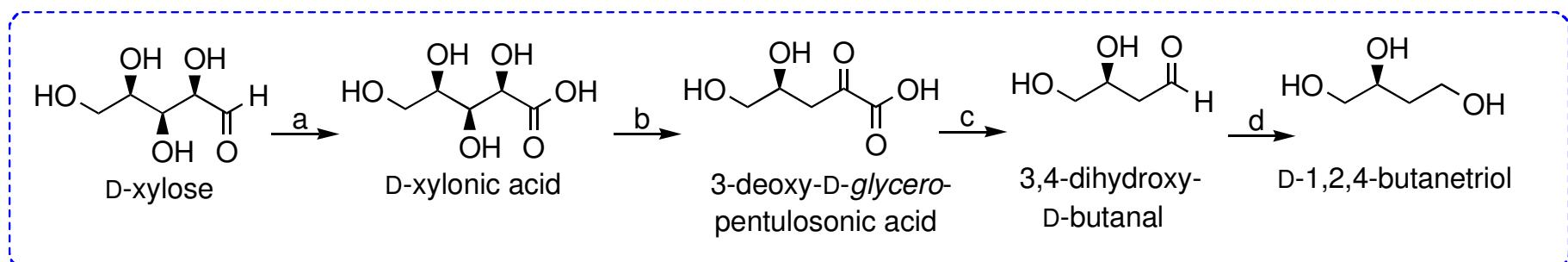
Elucidation of the *Escherichia coli* D-Xyloonic Acid Catabolic Pathway



Microbial Synthesis of D-1,2,4-Butanetriol from D-Xyloonic Acid Under Fermentor-Controlled Conditions

entry	<i>E. coli</i> construct	plasmid inserts	xyloonic acid	BT titer	BT yield
1	W3110 <i>serA</i> pWN7.126	$P_{tac} mdIC$ <i>serA</i> <i>lacI^Q</i>	18 g	0.08 g/L	1 %
2	W3110 <i>serA ΔyjhH ΔyagE</i> pWN7.126	$P_{tac} mdIC$ <i>serA</i> <i>lacI^Q</i>	28 g	8 g/L	45 %

Synthesis of D-BT from D-Xylose

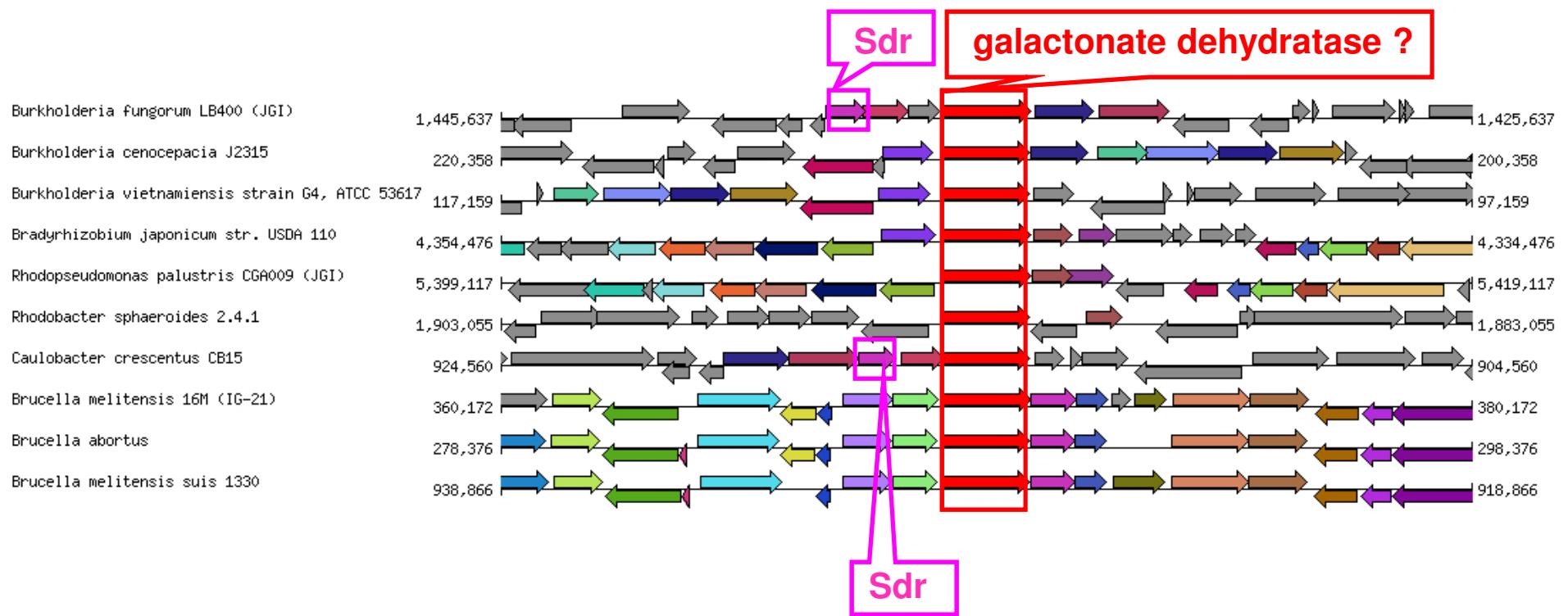


- a. D-xylonate dehydrogenase
- c. benzoylformate decarboxylase (*P. putida*)

- b. D-xylonate dehydratase (*E. coli*)
- d. alcohol dehydrogenase (*E. coli*)

Identification of a D-Xylose Dehydrogenase

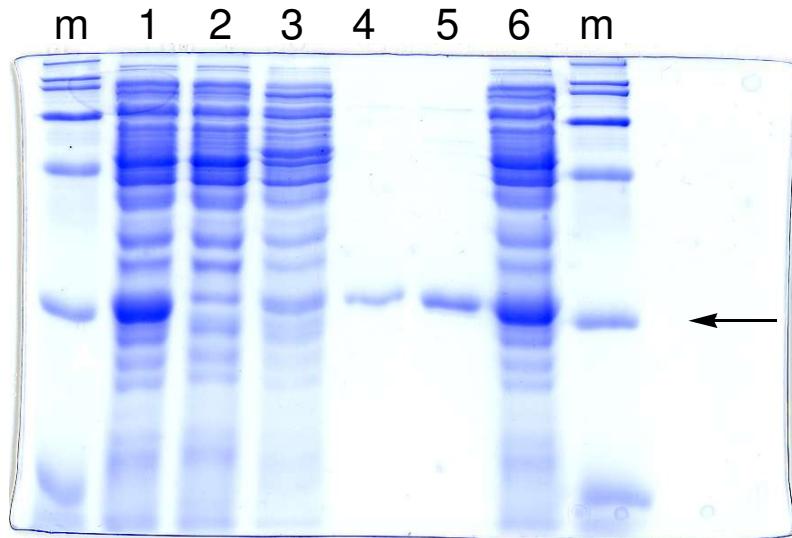
- BLAST search the ERGO database using the partial amino acid sequence of the previously isolated *P. fragi* D-xylonate dehydratase revealed Orfs with 50-70% of sequence identity.



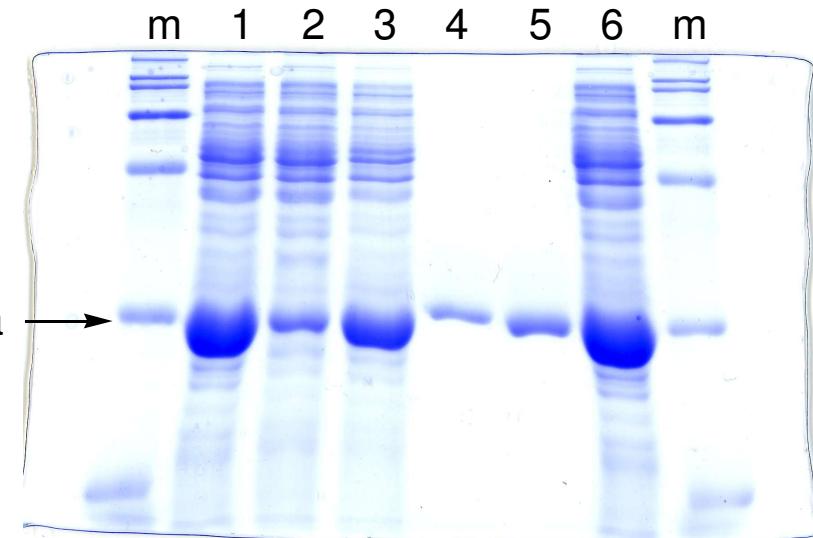
Characterization of Putative D-Xylose Dehydrogenases

- The *xdh* gene from *Burkhoderia fungorum* LB400 and the *xdh* gene from *Caulobacter crescentus* CB15 were respectively cloned into vector pQE-30. The two D-xylose dehydrogenases were expressed in *E. coli* and purified as 6xHis-tagged fusion proteins.

• SDS-PAGE for 6xHis-Xdh-LB400



• SDS-PAGE for 6xHis-Xdh-CB15



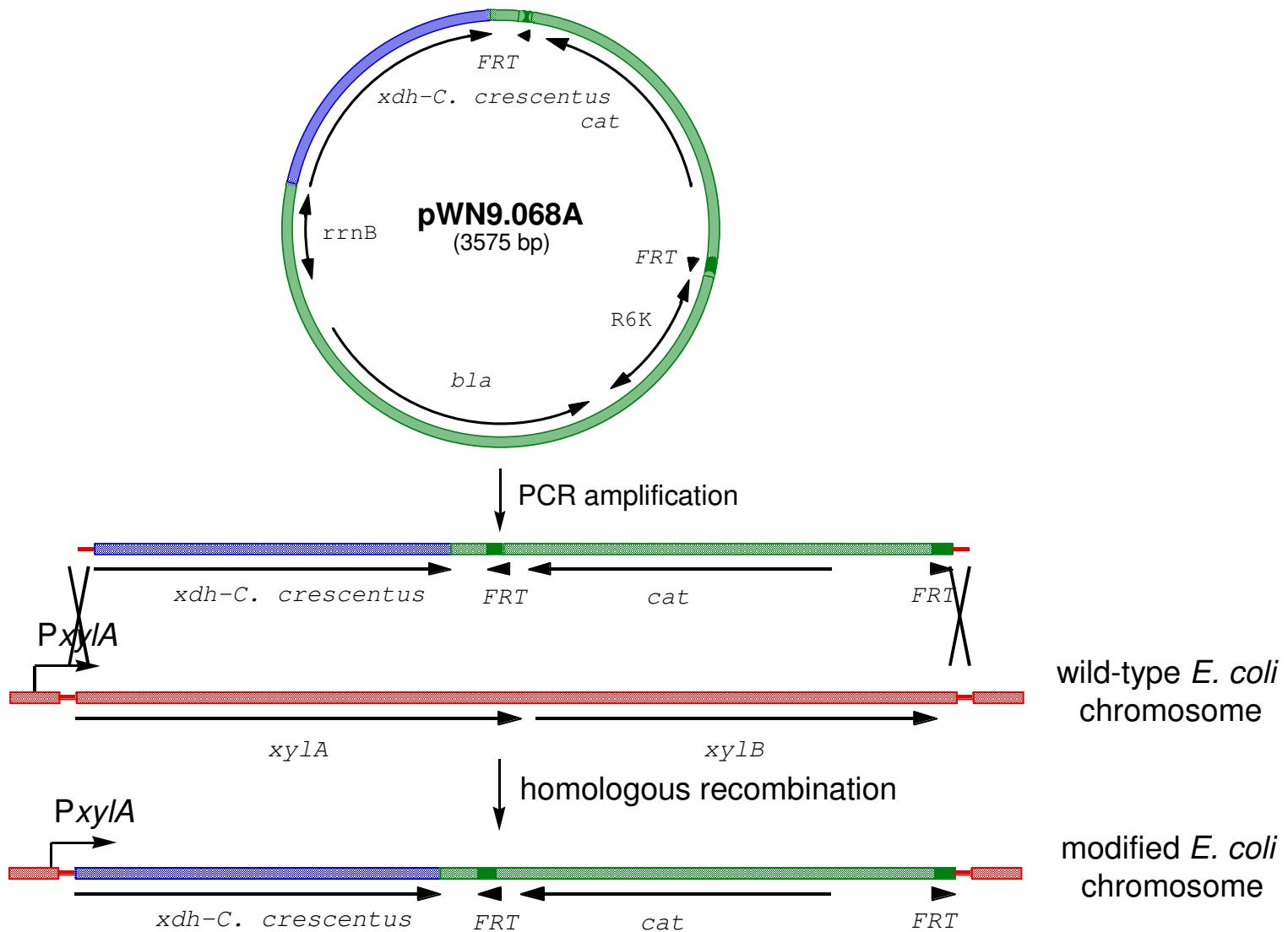
m, molecular weight markers; 1, cell lysate; 2, flow-through; 3, wash; 4, elution 1; 5, elution 2; 6, cell lysate.

Characterization of D-Xylose Dehydrogenases

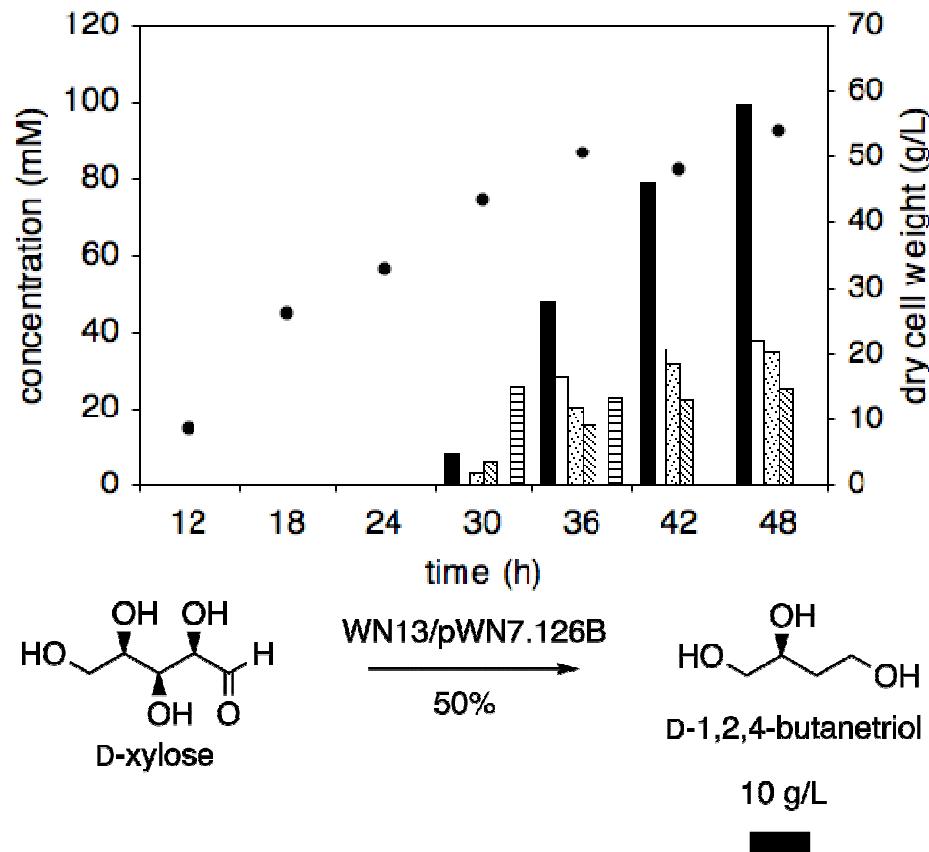
	NAD	D-xylose		D-glucose		L-arabinose	
	K_m (mM)	K_m (mM)	k_{cat}^c (s ⁻¹)	K_m (mM)	k_{cat} (s ⁻¹)	K_m (mM)	k_{cat} (s ⁻¹)
Xdh- <i>B. fungorum</i>	0.26 ^a	0.97	29	176	12	43	13
Xdh- <i>C. crescentus</i>	0.13 ^a	0.099	41	538	24	34	40

D-fructose, D-galactose, D-mannose and D-ribose were not substrates for either *B. fungorum* or *C. crescentus* xylose dehydrogenase

Insertion of *C. crescentus* *xdh* into the *E. coli* Chromosome



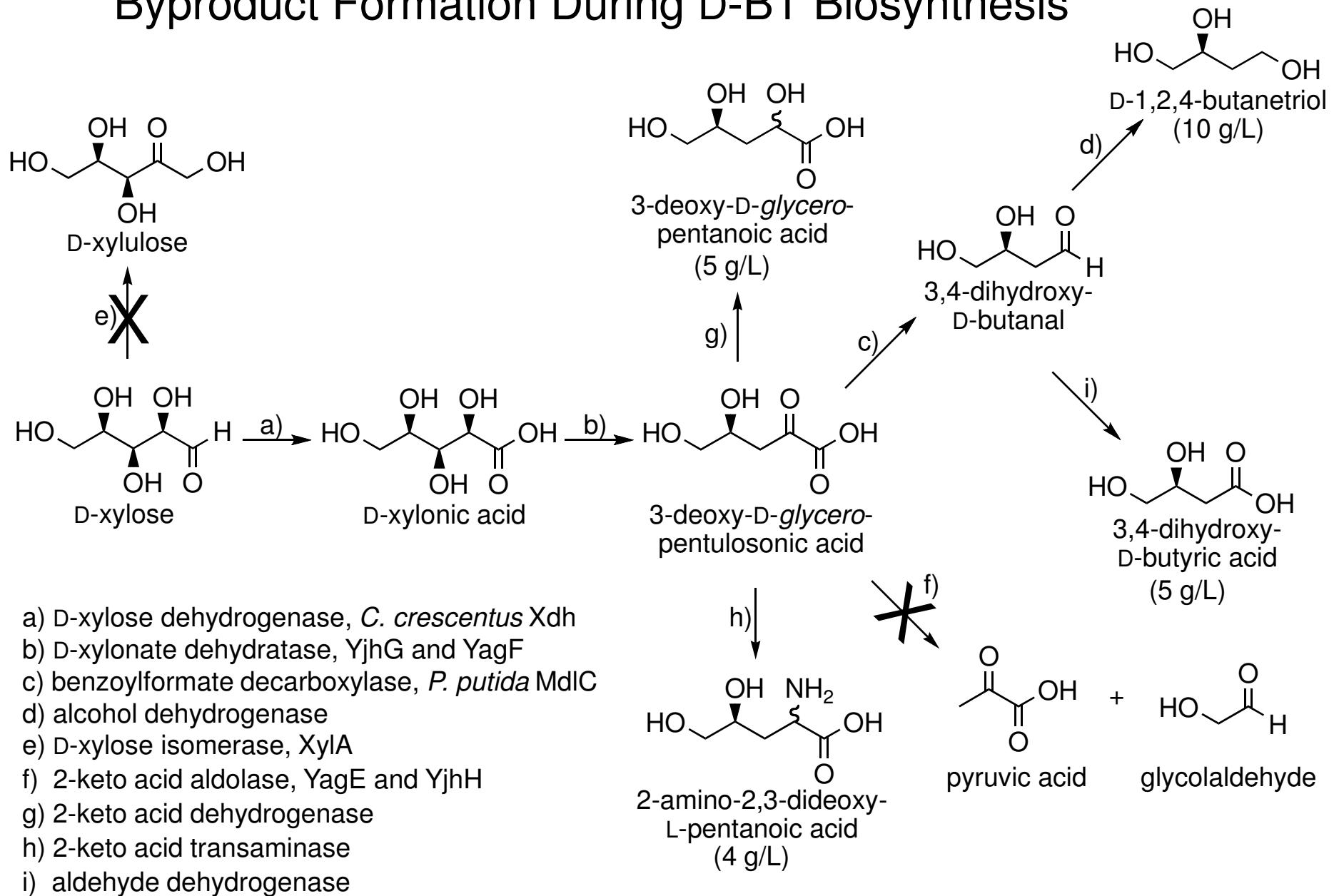
Fermentor-Controlled Synthesis of BT from Xylose



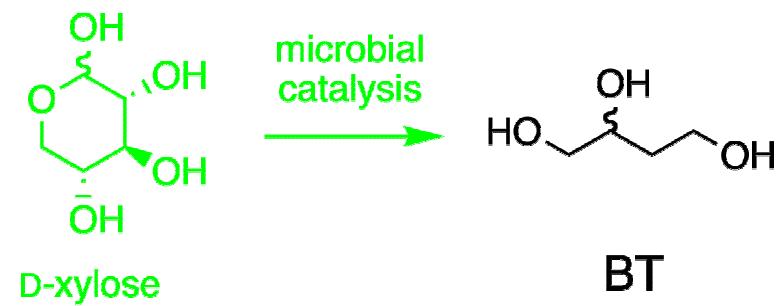
WN13: *E. coli* W3110~~serA~~*yjhH*~~yagE~~*xyIAB::xdh*-Cm^R

pWN7.126B: *serA*, *lacIQ*, *P_{tac}* *mdIC*

Byproduct Formation During D-BT Biosynthesis



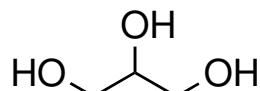
Green Synthesis of BT



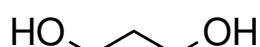
- nontoxic, renewable xylose
- reduction of salt waste streams
- avoidance of elevated temperatures and pressures
- single step
- domestic manufacture

Dual-Use Markets

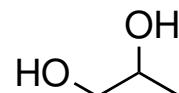
C-3 Biobased



glycerol



1,3-propanediol

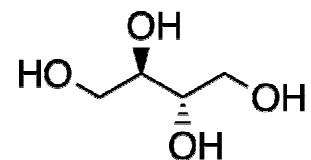


1,2-propanediol

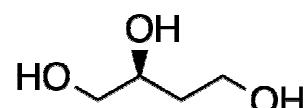
C-4 Biobased



1,4-butanediol



erythritol



1,2,4-butanetriol